ANTIMITOTIC SUBSTANCES FROM THE OVARIES OF VERTEBRATES[1]

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More and more evidence has been accumulated to show that the protoplasmic colloid is in a state of dynamic equilibrium between the factors which tend to cause it to gel or clot and those which tend to prevent or reverse this gelation. For a review of much of this evidence, see Heilbrunn (1956).

Substances which are potent in preventing protoplasmic clotting can be obtained from the ovaries of various animals (Heilbrunn, Chaet, Dunn and Wilson, 1954; Heilbrunn, Wilson and Harding, 1951). The fact that the ovaries of some fishes contain such substances has already been noted. In this paper, additional data will be presented, not only for the ovaries of fishes, but also for the ovaries of other vertebrate animals. These substances which prevent protoplasmic clotting also prevent the mitotic gelation which is a necessary precursor of the mitotic spindle; hence such substances have an antimitotic action.

The methods used and the plan of attack were much the same as in the earlier work. Table I shows the results obtained in the summer of 1953. Ovaries from 8 species of fishes all contained substances which had a strong antimitotic action on the eggs of the worm *Chaetopterus pergamentaceus*. The extracts were prepared by homogenizing the ovaries in sea water which had been made acid by the addition of HCl in sufficient amount to break down the buffers of the sea water. Following homogenization, within a few minutes the extracts were centrifuged (at 16,000 g) until most of the solid material was removed, and the supernatant fluid was then brought to a pH of approximately 8, that is to say, to the pH of sea water. In the case of the ovaries of *Sphaeroides maculatus*, the extracts were made in ordinary sea water rather than in acid sea water.

Two minutes after they had been inseminated, Chaetopterus eggs were introduced into the ovarian extracts, used either full strength, or diluted one-half or one-fourth with sea water. The eggs were kept at a temperature of 21° C. In all cases, the cleavage, if it occurred at all in the extracts, was delayed. Cleavage counts were made after a time interval long enough to insure that the percentage of cleavage was as high as it ever would be. In some instances, a cleavage furrow would start to form, but then it would disappear and the egg would become spherical again. Viscosity determinations were made 30–40 minutes after insemination. At this time, normal eggs show increased protoplasmic viscosity. Earlier work (Heilbrunn and Wilson, 1948) had shown that on our arbitrary scale, the viscosity of the protoplasm rises from 7 to 14. This means that it takes 14 seconds, for a cen-

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trifugal force of approximately 2200 g to move the granules of the protoplasm a sufficient distance so that the egg appears to be divided into zones. At this time, then, there is a gelation of some part of the protoplasm; this we call the mitotic gelation. In the control eggs such a gelation was always present. On the other hand, the protoplasm of the eggs exposed to the ovarian extracts always retained its original fluidity, and in some cases at least became even more fluid. This is indicated by the viscosity values shown in the table (Table I). In making these viscosity determinations, it was often not possible to make enough tests to be cer-

TABLE I

Effects of extracts of fish ovaries on cleavage of Chaetopterus eggs

Species	Extract no.	Grams ovaries	Ml. sea water	Viscosity 30–40 min. after insem.	% cleavage	Control % cleavage
Sphaeroides maculatus (Bloch and Schneider) puffer	1	72	100	6	0	100
Fundulus heteroclitus (Linnaeus) mummichug	6	10	25	4	0	97
	6	10	50	6	50	97
	6	10	100		46	97
	32	2	10	8	39	98
Opsanus tau (Linnaeus) toadfish	7	30	60	6 or less	0	99
	7	30	120	6 or less	0	99
Tautogolabrus adspersus (Walbaum) cunner	8	3	15	6	9	100
	33	1	9	8 or less	42	98
Tautoga onitis (Linnaeus) tautog	20	12	24	6	46	94
	29	15	30	8	15	100
Stenotomus versicolor (Mitchill) scup	22	2	10	8 or less	35	98
	22	2	20		37	98
	22	2	40		75	98
	31	2	10	6 or less	22	97
Anguilla rostrata (LeSueur) eel	35	6	30	8	49	89
Lophius americanus (Cuvier and Valenciennes) goosefish	4	100	100		0	93
	4	100	200		27	93

tain of the exact value for the viscosity, but in every case it was clear that the viscosity of the treated eggs was much less than that of the control (untreated) eggs centrifuged at the same time. These control eggs showed no indication of zones when they were centrifuged at the same force and for the same time as the treated eggs; whereas the eggs treated with a sufficient concentration of the ovarian extract, showed zones clearly. These treated eggs regularly gave viscosity values of 8 or less.

In the summer of 1954, the work on fish ovaries was continued. We made extracts from the ovaries of 7 other species of fish, and we also made new extracts from the ovaries of the toadfish (*Opsanus tau* L.). In the 1954 work, for each

gram of ovary, two ml. of sea water were used to obtain an extract. In the tests, the extracts were used full strength, half strength (that is to say, diluted with an equal volume of sea water), one-fourth strength, and one-eighth strength. In all cases the pH of the extract was adjusted so that it was approximately the same as that of sea water. The results of these 1954 experiments with fish ovaries are shown in Table II. Because of the fact that in most cases extracts weaker than half strength had but little effect, results obtained with these dilute extracts are not included.

Actually, our extracts always contain a mixture of substances which inhibit clotting and substances which promote clotting. If the extracts are allowed to age, the anti-clotting substances seem to lose their potency before the clotting substances do. Hence in extracts kept for a day or two in the icebox, the anti-clotting effect is reduced. Moreover, in one experiment done on the ovaries of fish (mackerel)

TABLE II

Additional data on the effect of extracts of fish ovaries on the cleavage of Chaetopterus eggs. The figures show percentage of eggs cleaving

Species	Full strength extract	Half-strength extract	Control
Brevoortia tyrannus (Latrobe) menhaden unripe ovary	10	17	100
Same—ripe ovary	1	4	100
Raja erinacea (Mitchill) skate—unripe ovary	0	48	97
Same—ripe ovary	0	80	97
Poronotus triachanthus (Peck) butterfish	96*	90	100
Opsanus tau (Linnaeus) toadfish	0	25	99
Centropristis striatus (Linnaeus) sea bass	10	81	99
Prionotus carolinus (Linnaeus) sea robin	76	86	97
Pseudopleuronectes americanus (Walbaum) winter flounder	11	96	100

* Cleavage was delayed for well over 6 minutes.

bought in a fish market, we found that the extracts caused clotting rather than liquefaction of the protoplasm. In every case, the ovarian extracts prepared from freshly caught fish kept the protoplasm fluid and prevented the mitotic gelation. This was determined by tests of viscosity made 30–40 minutes after insemination.

Table III shows the effect of extracts of ovaries from vertebrates other than fishes. These extracts also have a strong antimitotic effect, and in general they also prevent the mitotic gelation. Only one experiment with cow ovaries is listed. As a matter of fact, in attempting to discover how to make more potent extracts, we did many experiments with extracts from cow ovaries. The results obtained will be reported later. Suffice to say here that we always found sea water extracts of cow ovaries to exert a strong antimitotic action. However, in making these extracts we found it advisable to mince the ovaries instead of homogenizing them. Also it was not necessary to acidify the sea water in order to obtain a satisfactory extract.

Our results show that the ovaries of various vertebrate animals all contain antimitotic substances. At the present time, we are trying to obtain these substances

TABLE III

Effect of extracts of the ovaries of various vertebrates on the cleavage of Chaetopterus eggs

Animal	Extract no.	Grams ovaries	Ml. sea water	Viscosity 30–40 min. after insem.	% cleavage	Control % cleavage
Frog (*Rana pipiens*)	18	10	20	3	0	—
Frog (*Rana pipiens*)	18	10	40	—	0	98
Frog (*Rana pipiens*)	28	10	30	2	0	100
Frog (*Rana pipiens*)	28	10	60	—	0	100
Frog (*Rana pipiens*)	28	10	120	—	41	100
Salamander (*Amblystoma punctatum*)	38	0.5	5	6	0	99
Salamander (*Amblystoma punctatum*)	38	0.5	10	—	73	99
Chicken	39	26	52	5	2	98
Rabbit	23	2	10	8+	92*	97
Dog	34	2	10	8	3	94
Pig		25	25	<8	0	99
Lamb		14	15	<8	0	99
Cow		90	90	<8	0	100

* Cleavage was delayed 8 minutes.

in relatively pure form, in the hope that they may be able to cause regression of tumors in mice. Preliminary experiments indicate that our crude extracts are capable of causing such a regression, but the results we have obtained thus far vary greatly, and much more work is needed (Heilbrunn, Lippman and Rutman, 1955).

It must not be thought that the ovary is the only organ that contains antimitotic substances. We have made extracts from liver, testis and muscle, and all of these extracts prevent cell division. This is indeed what we would expect, if as we have said, protoplasm usually, perhaps universally, contains substances which have an inhibiting effect on protoplasmic clotting.

SUMMARY

1. Extracts from the ovaries of 14 different species of fishes all have an antimitotic effect on the eggs of the worm Chaetopterus.

2. Extracts from the ovaries of the frog, salamander, chicken, rabbit, dog, lamb, pig, and cow act in similar fashion.

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